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Mechanism of action of pulsed high electric field (PHEF) on the membranes of food-poisoning bacteria is an 'all-or-nothing' effect

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Abstract

Salmonella typhimurium (CRA 1005) was more sensitive than *Listeria monocytogenes* (NCTC 11994) to pulsed high electric field (PHEF) treatment in distilled water (10, 15 and 20 kV/cm), 10 mM tris-maleate buffer pH 7.4 (15 kV/cm) and model beef broth (0.75% w/v; 15 kV/cm). Sublethal injury could not be detected using a selective medium plating technique, indicating that bacterial inactivation by PHEF may be an 'all-or-nothing' event. PHEF-induced membrane permeabilization resulted in increased UV-leakage and a decreased ability of *L. monocytogenes* to maintain a pH gradient.

Keywords: Pulsed high electric field; *Salmonella typhimurium*; *Listeria monocytogenes*; Internal pH; Membranes

1. Introduction

As the search continues for novel, commercially viable, non-thermal food processing techniques, attention is beginning to focus on the possibility of using pulsed high electric field (PHEF) treatment. Detailed studies on the effects of homogeneous electric fields on microorganisms began with Sale and Hamilton (1967) who demonstrated that microbial inactivation was non-thermal and that it exerted its lethal effect by causing irreversible loss of membrane function. It is now generally accepted that the application of short electric field pulses (ns–ms) leads to the permeabilization of biological membranes, with the effect being reversible or irreversible depending on the intensity of the electric field and number of pulses applied (Castro et al., 1993).

2. Materials and methods

2.1. Growth of bacterial cultures

The two bacteria used in these investigations were *Listeria monocytogenes* (NCTC 11994) and *Salmonella typhimurium* (CRA 1005). Experimental cultures were prepared by inoculating tryptone soya broth (Oxoid, CM131) containing 0.6% (w/v) added yeast extract (Oxoid, L21) (TSBYE) followed by

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incubation at 37°C until cultures reached the late logarithmic/early stationary phase of growth. The bacteria were harvested by centrifugation and washed twice in 10 mM tris-maleate buffer pH 7.4 (TMB) and re-sedimented.

2.2. Pulsed high electric field treatment

L. monocytogenes and *S. typhimurium* were each treated in three suspending media: distilled water, tris-maleate buffer pH 7.4 (TMB) and a model beef broth (MBB) (0.7% (w/v), BBL® beef extract V, Becton Dickinson, USA). Each medium was inoculated to a final concentration of $\sim 1 \times 10^{10}$ bacteria/ml.

Aliquots of bacterial cell suspension were transferred to a static cell treatment chamber, consisting of two circular parallel stainless steel electrodes (30 mm diameter, previously cooled on ice) enclosed within a perspex casing. The treatment chamber was placed inside a purpose-built PHEF generator (EA Technology Ltd., Capenhurst, Chester, UK). Samples suspended in distilled water were treated at 10, 15 or 20 kV/cm for up to 10,000 pulses (exponential decay pulse wave form; pulse width = 50 μs), whereas samples suspended in TMB or MBB were treated only at 15 kV/cm (square-wave pulses; pulse width = 3–6 μs). The pulse rate was fixed at ~30 pulses per second and the applied voltage was monitored continuously. After every 2000 pulses the treatment chamber was removed from the PHEF generator and cooled on ice for 3 min and the temperature measured to ensure that it remained below 40°C.

2.3. Enumeration of survivors

After treatment, appropriate decimal dilutions in peptone diluent (0.1% peptone + 0.85% NaCl) were prepared from each sample. The number of *L. monocytogenes* survivors were enumerated by spotting triplicate 0.05-ml volumes onto tryptone soya agar (Oxoid CM131) with 0.6% added yeast extract (TSAYE), TSAYE + NaCl (3% w/v) and *Listeria* Selective Agar (OLSA; Oxford Formulation containing OLSA base [Oxoid CM856] plus *Listeria* Selective Supplement [Oxoid SR140E]). The number of *S. typhimurium* survivors was enumerated by spotting triplicate 0.05-ml volumes onto TSAYE, TSAYE + NaCl (3% w/v) and Xylose Lysine Deoxycholate (XLD; Oxoid CM469). Plates were incubated for 48 h at 37°C. The numbers of sublethally injured bacteria were taken as the difference between counts obtained on TSAYE and those obtained on the selective agars (TSAYE + 3% NaCl, OLSA and XLD).

2.4. Leakage of UV absorbing substances

Samples (2.8 ml) were centrifuged at $10{,}000 \times g$ for 10 min (Sorvall, RC5C). The upper 1 ml of the supernatant was removed and the UV absorbance was measured at a wavelength of 280 nm (CE 2020, Cecil Instruments Ltd., Cambridge, UK).

2.5. Internal pH

The effect of PHEF on the internal pH of *L. monocytogenes* was measured using an intracellular fluorescent probe, following the method of Breeuwer et al. (1996).

3. Results

3.1. Viable counts

Treatment in distilled water at 10 kV/cm had no effect on viable counts of *L. monocytogenes* (Fig. 1) or *S. typhimurium* (Fig. 2). However treatment with 10,000 pulses at 15 kV/cm resulted in an approximately 1-log cycle decrease in the numbers of *L. monocytogenes* and a greater than 2-log reduction in the numbers of *S. typhimurium*.

S. typhimurium was also more sensitive to pulsed high electric field (PHEF) than *L. monocytogenes* at 20 kV/cm, with treatment at 10,000 pulses bringing about approximately 4- and 6-log cycle reductions in the viable counts of these microorganisms, respectively. For both *L. monocytogenes* and *S. typhimurium* there was no difference between the counts on non-selective and selective plating media at all field strengths. Treatment of both microorganisms in 10 mM tris-maleate buffer pH 7.4 or model beef broth had no significant effect on their survival or the level of injury (data not shown).

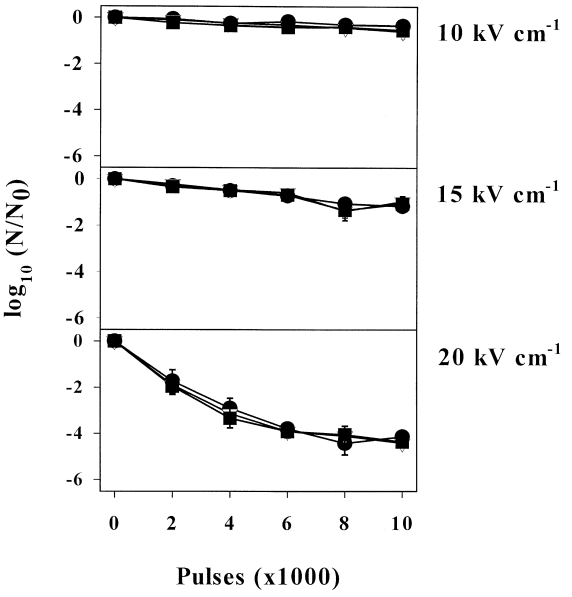

Fig. 1. The effect of PHEF treatment at 10, 15 and 20 kV/cm in distilled water on the survival of *L. monocytogenes* (NCTC 11994). (●, TSAYE; ▽, TSAYE + NaCl; ■, OLSA).

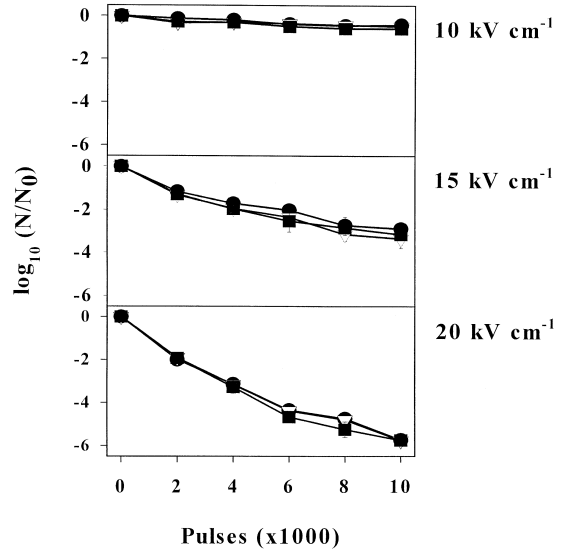

Fig. 2. The effect of PHEF treatment at 10, 15 and 20 kV/cm in distilled water on the survival of *S. typhimurium* (CRA 1005). (●, TSAYE; ▽, TSAYE + NaCl; ■, XLD).

3.2. Leakage of UV-absorbing substances

Generally, increasing the field strength and number of pulses resulted in increasing levels of leakage of UV-absorbing substances from both microorganisms when treated in distilled water (data not shown). Comparable levels of UV leakage were observed for *L. monocytogenes* and *S. typhimurium* at both 10 kV/cm and 15 kV/cm. At 20 kV/cm, however, the levels of UV-absorbing substances from *S. typhimurium* were found to be approximately four times those observed for *L. monocytogenes*. Treatment of both bacteria in 10 mM tris-maleate buffer pH 7.4 had no effect on the overall levels of UV leakage observed (data not shown).

3.3. Maintenance of internal pH

The ability of *L. monocytogenes* to maintain internal pH was unaffected when treated at 10 kV/cm with up to 10,000 pulses in distilled water. However, treatment at both 15 and 20 kV/cm resulted in significant decreases in ΔpH after exposure to 2000 pulses, and the pH gradient was almost entirely dissipated after treatment with 4000 pulses at 20 kV/cm.

When 10 mM tris-maleate buffer or beef broth were used as suspending media, treatment resulted in a significant decrease in internal pH similar to that seen following treatment in distilled water.

4. Discussion

Viable counts were carried out using non-selective TSAYE and two selective agars to provide an estimate of the degree of sublethal injury caused by PHEF treatment. However, for any particular treatment, no differences were found between counts on non-selective and selective agars at all field strengths and number of pulses applied, in all suspending media. It has been demonstrated previously, when a similar technique was applied for the enumeration of high pressure-treated *L. monocytogenes* cells, that the percentage of survivors sustaining sublethal injury increases with increasing severity of treatment (Simpson and Gilmour, 1997). Inactivation by PHEF may be an ‘all or nothing event’ in which little or no sublethal injury is sustained before cells become completely inactivated. Alternatively, the selective plating method may be inadequate for the detection of sublethal injury induced by PHEF treatment.

It is generally accepted that PHEF exerts its lethal

effects primarily by causing the formation of pores in the cytoplasmic membrane of treated cells (Castro et al., 1993; Wouters and Smelt, 1997). This is supported by the present study in which increasing severity of treatment led to greater leakage of UV-absorbing materials.

The ΔpH results obtained for the treatment of *L. monocytogenes* in distilled water at 10 kV/cm show that this non-lethal field strength had no effect on the ability to maintain a pH gradient. This provided further evidence that bacteria were not significantly or irreversibly permeabilized at this field strength. However, when *L. monocytogenes* was treated at 15 kV/cm in distilled water, tris-maleate buffer or model beef broth its ability to maintain a pH gradient was significantly impaired, corresponding to the onset of bacterial inactivation and significant increases in UV-leakage.

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